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IMMUNOLOGICAL NOTES

I.-VII.

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WE have attempted in a number of main papers and a series of short notes to bring up to date the development of our work on immunity. In work of this description there are always many completed experiments that have not been linked up with other lines of the work. We feel that these results may be of sufficient interest to other workers in this field to justify publication. We have therefore collected, in addition to the main papers, a series of short notes on immunity which, although apparently unconnected, may all later take their places in the chain of evidence leading to a better knowledge of the mechanism of immunity response.

In the present series of notes we see indications of how a minute but repeated stimulus of toxin may produce immunity; there is an obvious link between this fact and naturally acquired immunity in man and in horses that cannot be traced to a stimulus sufficient to cause clinical disturbance. We have some evidence that certain antigens act as stimuli when given in widely different doses and some evidence is also available that dissociation of toxin-antitoxin mixtures occurs; it may be that here we have a partial explanation of the success attending the use of 1/10 L+ mixtures by Park and Zingher; it may also be linked up with the low immunising efficiency of unneutralised toxoid given intravenously, although partially neutralised toxoid given by the same route is successful. The conclusion suggests itself that the antigenic response to a given number of binding units depends upon the manner in which the stimulus is presented, the route by which it is given and the extent of its specific toxicity; in addition a "crowding out" of response may occur or a non-specific stimulus may exist.

Our knowledge of immunity in general must gain from a detailed study of one section such as immunity to diphtheria; our aim has been to study and link together the factors involved in the preparation of toxin, its modification and its destruction, and its mode of action as a stimulus to antibody production.

I.—VARIATION IN RESPONSE OF INDIVIDUAL GUINEA-PIGS TO IMMUNISATION.

There are differences in the response given by individual guinea-pigs to the injection of an antigen. Careful selection of uniform healthy animals lessens the amount of variation.

It will be seen that the guinea-pigs usually became Schick negative between the 14th and 17th day; this occurred on 32 occasions while 5 guinea-pigs were immune earlier than 14 days and 15 later than the 17th day. There is marked variation in different guinea-pigs injected on the same day, and even greater variation occurs between groups of guinea-pigs injected on different days.

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* Some of this work has been interwoven to some extent with investigations carried out in the past in conjunction with Dr Sudmersen, Miss Allen, Miss Hopkins and Miss Attwood who are no longer working in this field.

TABLE I.

Showing the number of days before guinea-pigs injected on different occasions with 5 *c.c. modified toxin* B. 1092 *became Schick negative.*

19th March	14	16	16	...	...	...
9th May	14	14	17	17	over 17	over 17
26th ,,	14	16	...	...	...	...
30th ,,	14	14	17	18	19	21
18th June	12	12	12	14	16	16
4th July	17	17	17	17	over 19	over 19
8th ,,	13	15	17	...	...	...
10th ,,	15	20	over 20	...	...	...
14th ,,	11	14	16	...	...	...
6th August	14	14	19	19	19	21
2nd September	17	17	20	22	...	...
23rd October	14	14	14	17	...	...

Although these results show considerable variation, this is not sufficient to render this method of testing antigenic values useless. The variation is always much smaller when it is possible to avoid using animals immediately after their arrival from outside sources. When the condition of guinea-pigs is kept as uniform as possible by selecting animals from the same source, uniform results can be obtained.

II.—VARIATION IN ANTIGENIC RESPONSE ACCORDING TO METHOD OF INJECTION.

Guinea-pigs show a greater immunity response to the same amount of diphtheria toxoid when it is injected intraperitoneally or injected subcutaneously in a dilute form than when injected subcutaneously or intracutaneously undilute, or intramuscularly either dilute or undilute.

Table II. shows the immunity index of 42 guinea-pigs injected with the same dose, 0·2 c.c. of a modified toxin B. 1092. The animals were divided into groups of 6 and each group was injected in a different way. Three weeks later, and thereafter at weekly intervals, the guinea-pigs received a Schick dose of toxin. The figure recorded for the immunity index of any animal is the number of the first Schick dose that failed to produce a positive reaction. It will be seen that the best results were obtained when the toxin was diluted and injected subcutaneously or intraperitoneally; undiluted toxin injected subcutaneously intracutaneously or intramuscularly was less efficient, but the absence of dilution made no difference when the toxin was injected intraperitoneally. The intramuscular injection of diluted toxin failed to immunise any of the 6 guinea-pigs.

TABLE II.

Showing the effect of the method of injection upon the immunity index of toxoid.

Modified toxin injected.	Route.	Immunity index of 6 guinea-pigs of each group.					
0·2 c.c. diluted to 5 c c.	intraperitoneal.	2	2	3	7	x	x
0·2 ,, undilute.	,,	2	3	3	5	7	x
0·2 ,, diluted to 5 c.c.	subcutaneous.	2	2	3	4	x	x
0·2 ,, undilute.	,,	6	7	7	7	x	one died.
0·2 ,, ,,	intracutaneous.	4	5	6	7	9	x
0·2 ,, ,,	intramuscular.	4	5	7	8	x	x
0·2 ,, diluted to 5 c.c.	,,	x	x	x	x	x	x

x indicates that a positive reaction still occurred at the 9th injection of Schick toxin.

The guinea-pigs were taken at random from stock accumulated from several sources at different times. At the time of the preliminary injections the weights of all the animals lay between 300 and 350 grms. In each group there was marked variation between the response of individual guinea-pigs. It has been our general experience in the past that there is a tendency for animals in poor condition to respond more feebly to an antigen. It has frequently happened that one guinea-pig in a small group has failed to respond as rapidly as the others, and often this is the animal that has shown least increase in weight during the period of observation. It would appear however that many other factors are involved as an analysis of our results show a tendency only towards any correlation between loss in weight and failure to respond. In the batch of guinea-pigs under review, the amount of modified toxin injected was insufficient to cause any local or constitutional disturbance, for 5 c.c. of the same toxin is tolerated by guinea-pigs without local reaction or loss in weight. The intramuscular injection of a large volume of fluid into guinea-pigs in the last group might possibly have had an ill effect. During the first week after injection several of these guinea-pigs lost weight and none showed an immunity response.

III.—THE INFLUENCE OF AGE ON THE RATE OF PRODUCTION OF IMMUNITY IN GUINEA-PIGS.

Young guinea-pigs tend to be less readily immunised than older animals.

It will be seen from table III. that the individual differences among guinea-pigs of the same weight are as great as the differences for guinea-pigs of various weights above 250 grms.

Another series recorded in table IV. of normal guinea-pigs of different weights was injected every week with Schick doses of toxin. The time taken before any guinea-pig was Schick negative varied considerably; the individual differences between guinea-pigs of the same weight was very great and no group appeared with certainty to be more rapidly immunised than any other although there was a slight tendency for heavy guinea-pigs to respond more rapidly.

TABLE III.

Showing the immunity index for 1·0 *c.c. doses of toxoid and toxin-antitoxin mixture injected into guinea-pigs of different weights.*

Weight of guinea-pigs.	Immunity index of toxoid.	Immunity index of T.A.M.
200	over 16	...
250	5	4
300	2	2
350	3, 4, 4	2, 2
400	4, 5	2
450	3, 4, 7	2, 3, 3
670	5	...
980	6	...

TABLE IV.

Showing the number of weekly Schick doses of toxin required to render immune groups of guinea-pigs of different weights.

Weight of guinea-pig (at commencement of experiment).	Immunity index.
200 grms.	over 17, 26, 38.
250 ,,	over 43.
300 ,,	34, 51.
350 ,,	over 20, 37, 45, over 50.
400 ,,	over 20, 25.
450 ,,	14, 22, over 30, over 35.
500 ,,	over 15, over 17, 18.
600 ,,	18, 28.

In the next series (see table V.) fully grown guinea-pigs were compared with young guinea-pigs; old guinea-pigs responded more readily than the young ones and rapidly became immune.

TABLE V.

Showing the number of weekly Schick doses of toxin required to render immune groups of guinea-pigs of different ages.

Weight of guinea-pig.	Age.	Immunity index.
240 to 290 grms.	...	20, over 22, 25, 31.
800 ,, 825 ,,	...	9, 10, 18.
820 grms.	2 years	13.
900 ,,	2 ,,	13.
1005 ,,	1½ ,,	13.

IV.—THE EFFECT OF THE ADDITION OF SMALL AMOUNTS OF TOXIN ON THE ANTIGENIC VALUE OF MODIFIED TOXIN.

The addition of sub-lethal doses of diphtheria toxin to fully modified toxin considerably increases the antigenic value as measured by the immunity index. There is some evidence to show that other toxic substances may act in the same way; in a single experiment the antigenic value of diphtheria toxoid was improved by the addition of B. Welchii *toxin.*

In a previous paper (Glenny, Hopkins and Pope 1924, this *Journal,* xxvii. 261) it was stated "that we had considerable evidence that, unit for unit, the antigenic value of a partially modified toxin was better than that of a toxin completely modified." This point was not considered in detail in the above paper as our main concern was to show that completely modified toxins were good antigens. In a recent paper Zingher and Park (1924, *Amer. Journ. Dis. Children,* xxviii. 478) have given their experiences with modified toxins, and they express the same view. In their conclusions they state "it is of advantage to have a slight excess of free toxin (from 1/15th to 1/20th minimal lethal dosage in each dose) in the toxoid."

TABLE VI.

Showing the antigenic value of toxoid with and without the addition of small volumes of toxin.

Dose of toxoid.	Amount of toxin added.	Number of guinea-pigs showing an immunity index of—				
		1.	2.	3.	4.	over 4.
0·1 c.c. . . .	nil	...	3	3	1	2
0·1 ,, . . .	0·001 to 0·003 c.c.	2	4	3	...	1
1·0 ,, . . .	nil	5	4	2	...	...
1·0 ,, . . .	0·001 to 0·003 c.c.	4	...	...	...	...

In the first experiment from 10 to 30 Schick doses of toxin were added to 0·1 c.c. of modified toxin. Before the addition of the toxin the toxoid produced no local reactions when injected subcutaneously into guinea-pigs; after the addition of toxin large reactions were produced but the animals survived. It will be seen from table VI. that a definite increase in antigenic strength resulted from the addition of toxin.

In a later experiment a more exact comparison was made; guinea-pigs were injected with 1 c.c. of toxoid to which had been added varying amounts of toxin. After an interval of 17 days the guinea-pigs were injected on alternate days with a Schick dose of toxin. It will be seen from table VII. that two out of three guinea-pigs injected with toxoid to which 20 Schick doses of toxin had been added were immune when first tested at the end of 17 days; with 10 Schick doses added, two out of three of the guinea-pigs were immune in 19 days; when 5 Schick doses were added, two out of three guinea-pigs were immune in 21 days; with still less toxin 27 days elapsed before two out of three guinea-pigs were immune.

The injection of the mixture of 1 c.c. of toxoid and 10 Schick doses of toxin produced very small swellings and slightly more toxic mixtures caused swellings of moderate size. The improvement in the antigenic value following the addition of these small amounts of toxin is much greater than would be expected if the amount of added toxin is considered in terms of binding units. The largest addition of toxin was equivalent to only 0·2 per cent. of the total combining power of the toxoid. It is possible that the increased antigenic strength of these slightly toxic preparations is due in some way to local damage.

TABLE VII.

Showing the effect on the antigenic value of toxoid of the addition of small amounts of toxin.

Volume of toxoid injected.	Volume of toxin added.	Volume of toxin in terms of Schick doses.	Number of guinea-pigs immune after various periods.						
			17 days.	19 days.	21 days.	23 days.	25 days.	27 days.	Over 27 days.
1·0	0·0001 c.c.	1	...	...	...	...	1	1	1
1·0	0·0005 ,,	5	...	...	2	1	...	...	...
1·0	0·001 ,,	10	1	1	...	1	...	...	...
1·0	0·002 ,,	20	2	1	...	...	...	...	...

This experiment was repeated with similar results (see table VIII.). The series of test doses of Schick toxin was started on the 10th day and earlier immunity resulted. The preliminary positive Schick reactions have a stimulating effect upon the immunity of the guinea-pigs and a series of guinea-pigs tested on alternate days starting on the 10th day (as in table VIII.) will be Schick negative earlier than a similar series started on the 17th day (as in table VII.).

If we consider as a convenient index of comparative antigenic efficiency the date upon which two out of three guinea-pigs were immune after a particular injection we see from table VIII. that 1·0 c.c. of toxoid without any added toxin or with one or two Schick doses added produces immunity in 19 days; with cobra venom added, in 17 days; with five or more Schick doses of toxin or with *B. Welchii* toxin added, a Schick negative condition is reached in 14 days, *i.e.* three days earlier than the 17 days needed to produce a similar degree of immunity with twice the amount of toxoid alone.

TABLE VIII.

Showing the effect on the antigenic value of toxoid of the addition of small amounts of diphtheria or B. Welchii *toxins and of sub-lethal doses of cobra venom.*

Volume of toxoid injected.	Amounts of toxin added.	Number of guinea-pigs immune after various lengths of time.					
		14 days.	17 days.	19 days.	21 days.	24 days.	Over 24 days.
nil	10 Schick doses diphtheria toxin .	...	...	...	...	...	3
nil	20 ,, ,, ,, .	...	...	...	...	...	1
1·0 c.c.	nil	...	1	2	...	...	...
2·0 ,,	nil	1	1	...	...	1	...
1·0 ,,	1 Schick dose diphtheria toxin .	...	...	2	1	...	...
1·0 ,,	2 ,, ,, ,, .	1	...	1	...	1	...
1·0 ,,	5 ,, ,, ,, .	2	...	...	1	...	...
1·0 ,,	10 ,, ,, ,, .	2	1	...	...	...	...
1·0 ,,	20 ,, ,, ,, .	1	...	...	...	...	...
1·0 ,,	1/5 M.L.D. cobra venom . .	...	3	...	...	...	...
1·0 ,,	1/5 M.L.D. (mouse) *B. Welchii* toxin	3	...	...	...	...	...

V.—THE ANTIGENIC VALUE OF MODIFIED TOXIN AFTER BOILING.

Modified toxins after boiling retain approximately 5 *per cent. or* 10 *per cent. of their original antigenic power. Some antigenic power still remains after boiling for five minutes.*

During the course of our work on toxin modified by formaldehyde it became of interest to compare the heat stability of the modified as compared with the unmodified toxin. For this purpose samples

TABLE IX.

Comparing the immunity index of various toxins and modifications before and after boiling.

Batch.	Index before heating.	Index after just boiling.	Index after boiling for a definite time.
Unmodified toxin J 3460	...	1·0 c.c. over 15	...
	...	5·0 ,, 7	...
PX 57 = J 3460 after modification	0·1 c.c. 3	...	...
	0·2 ,, 3	0·2 c.c. 10	...
	0·5 ,, 1 to 2	0·5 ,, 13	...
	1·0 ,, ,,	...	...
	...	2·0 ,, 3	...
	5·0 ,, 1	5·0 ,, 1, 2	5·0 c.c. boiled 1 min. 2
	...	...	5·0 ,, ,, 5 ,, 2, 9
	...	...	5·0 c.c. boiled 15 min. over 15
Partially modified and concentrated toxin WSC 14 A.	0·01 c.c. 2, 4	...	...
	0·05 ,, 2, 3	...	...
	...	0·2 c.c. 6	...
	...	0·5 ,, 3	...
	...	1·0 ,, 2	...
	...	2·0 ,, 1	...

of the original toxin and of the modified toxin were heated to boiling point under standard conditions, so that the time required to reach the boiling point did not vary more than a few seconds between various samples. Further samples were boiled for a definite length of time, in which case a reflux condenser was used in order to keep the volumes constant. The toxin used was J 3460, and its antigenic value is recorded after modification with formaldehyde under the key number PX 57. This toxin was modified by the addition of 0·4 per cent. formaldehyde and incubation at 30° C. for 11 days. Its P_H was then 5·5. Table IX. shows that the toxin modified with formaldehyde retains its antigenic properties to a much greater extent after boiling than does the unmodified toxin.

Further experiments were carried out with a series of modified toxins, prepared from the same original toxin by adding increasing amounts of formaldehyde.

TABLE X.

Showing the immunity index of various modifications of a toxin before and after boiling.

Key No.	Percentage of formaldehyde added.	Volume injected.	Index before boiling.	Volume injected.	Index after boiling.
PX 148	0·1	0·02 c.c.	4	1·0 c.c.	5
PX 150	0·3	0·02 ,,	6	1·0 ,,	5
PX 150	...	...	...	5·0 ,,	2
PX 151	0·4	0·05 ,,	2, 2, 3, 4	1·0 ,,	6
PX 152	0·5	0·05 ,,	3, 3, 7	1·0 ,,	over 8
PX 152	...	...	...	2·0 ,,	4
PX 152	...	...	...	5·0 ,,	2
PX 153	1·0	0·05 ,,	6, 6, 6	1·0 ,,	over 8
PX 153	...	...	...	2·0 ,,	3
PX 153	...	...	...	5·0 ,,	5

It will be seen that there is a considerable decrease in the antigenic values after boiling but injections of 5·0 c.c. of the boiled material can still produce good immunity.

VI.—THE ANTIGENIC VALUE OF PARTIALLY NEUTRALISED TOXOID.

The antigenic value of modified toxin is not greatly reduced by partial neutralisation with antitoxin.

We have already shown (Glenny Hopkins and Pope 1924, this *Journal*, xxvii. 261) that "in an immunising mixture prepared with modified toxin the amount of antitoxin present does not, within wide limits, affect the antigenic power."

Further experimental evidence is given below. A batch of concentrated toxoid with and without antitoxin was injected into guinea-pigs; the antigenic values determined by weekly Schick doses are recorded in table XI.

TABLE XI.

The effect of adding antitoxin to concentrated toxoid.

Number of units of antitoxin added to each c.c. of concentrated toxoid.	Antigenic values of—	
	1·0 c.c.	5·0 c.c.
none	1, 1	1, 1
1	1, 4	1, 1
2·5	2, 3	1, 7
5	3, 4	1, 3
10	1, 4	1
25	7, over 7	7, over 7
50	6	2, 7

It is only when very large quantities of antitoxin are added that the antigenic value tends to fall.

A further group of guinea-pigs injected with the same mixtures as above was tested with Schick doses of toxin every two days starting on the ninth day after the original injection. Few animals were used, but the results suggest that but little reduction in antigenic value is caused by the addition of antitoxin to concentrated toxoid.

TABLE XII.

Showing the effect of adding antitoxin to concentrated toxoid.

Volume of concentrated toxoid.	Units of antitoxin.	Schick reactions.					
		9 days.	11 days.	13 days.	16 days.	21 days.	23 days.
1·0 c.c.	none	+	+	+	−		
1·0 ,,	1 unit	+	+	+	−		
1·0 ,,	2·5 units	+	+	+	+	+	+
1·0 ,,	5·0 ,,	+	+	+	+	−	
1·0 ,,	10·0 ,,	+	+	+	+	−	
1·0 ,,	50·0 ,,	+	+	+	+	+	+
5·0 ,,	none	+	+	+	−		
5·0 ,,	5·0 units	+	+	+	+	−	
5·0 ,,	12·5 ,,	+	+	+	+	−	
5·0 ,,	25 ,,	+	+	+	−		
5·0 ,,	50 ,,	+	+	+	+	−	
5·0 ,,	125 ,,	+	+	+	+	+	−
5·0 ,,	250 ,,	+	+	+	+	+	+

The signs in each line represent the results of successive Schick tests on one guinea-pig.

VII.—IMMUNISATION WITH SMALL AMOUNTS OF TOXIN.

By the daily injection of very small amounts of toxin, in all less than 3 M.L.D., guinea-pigs have been rendered immune (Schick negative) in 22 days.

Glenny and Allen (1922, *Journ. Hyg.*, xxi. 104) have already shown that so small an amount of diphtheria toxin as the Schick dose (the equivalent of 0·001 unit of antitoxin) will act as a secondary stimulus.

The effect of small doses of toxin as a primary stimulus is worthy of attention; immunity apparently may be produced in man without any recognisable clinical disturbance. It would seem therefore that small doses of toxin suitably presented may induce primary immunity. Glenny and Hopkins (1923, *Brit. J. Exp. Path.*, iv. 283) have shown that a guinea-pig may become immune (Schick negative) within 9 weeks when injected at weekly intervals with a Schick dose of an old matured toxin. The total toxin injected was 0·0036 c.c. containing in all less than one-fifth of an M.L.D. With a less mature toxin (J 3284) 20 weeks was the shortest time in which a guinea-pig became immune, and the majority of guinea-pigs became immune after receiving a total amount of toxin equivalent to only 1 M.L.D. spaced at weekly intervals of over 30 weeks. A few further experiments may be recorded: in all cases the same toxin (J 3284) was used for the Schick toxin.

Four guinea-pigs were injected with living virulent *C. diphtheriæ* (5 million organisms). Four weeks later a weekly series of Schick doses was begun. Two guinea-pigs became Schick negative in 17 and 31 weeks. The other two were not immune after 16 and 37 weeks when discarded. In this experiment we failed to produce any good degree of immunity with living cultures. In the next series of tests guinea-pigs were injected with minute doses of toxin spaced over the whole surface of the animal and after a period of 4 or 8 weeks the routine Schick testing was commenced.

We have found in other experiments that ordinary guinea-pigs injected each week with a Schick dose of toxin have become immune in 20, 23, 25, 31, 43 and 44 weeks, while two were not immune when discarded after 22 and 32 weeks. In the series shown in table XIII. the guinea-pigs which received the primary stimulus and were retested after a rest became immune more rapidly than the animals which had no rest; it is difficult, however, to attach much significance to such a result unless large numbers of tests are done.

TABLE XIII.

Total toxin injected as primary stimulus in terms of Schick doses.	Number of injections given at the same time.	Amount of toxin injected at each point in terms of Schick doses.	Rest.	Immunity index.
0·01	1	0·01	8 weeks	12
0·05	1	0·05	8 ,,	12
0·2	20	0·01	8 ,,	over 17
0·2	20	0·01	4 ,,	20
0·4	40 (during 3 days)	0·01	4 ,,	over 37
1·0	20	0·05	8 ,,	15
1·0	20	0·05	8 ,,	over 14
1·0	20	0·05	4 ,,	16

In the next experiment single small doses were given.

TABLE XIV.

Toxin injected as primary stimulus in terms of Schick doses.	Rest.	Immunity index.
0·001	4 weeks	15, 18
0·001	8 ,,	over 42 (*a*), over 101 (*b*)
0·01	4 ,,	24, 48
0·1	4 ,,	over 28
0·1	8 ,,	39
1·0	4 ,,	15 (immunity confirmed by antitoxin value of blood, 0·1 unit per c.c.)

It is of interest to note how refractory some guinea-pigs may apparently be; several animals already recorded have remained persistently Schick positive for over 40 weeks although an average guinea-pig may become immune in 20 weeks by this treatment. The guinea-pig marked (*a*) above did not show antigenic response to the intradermic injections of Schick toxin but when injected subcutaneously with a toxin antitoxin mixture after the 42nd positive reaction the animal responded in a normal manner. One guinea-pig (*b*) was continually injected with Schick toxin for 101 weeks and remained non-immune. The absence of immunity was checked by a test upon the antitoxic titre of the blood; no antitoxin (*i.e.* less than 0·0005 unit per c.c.) could be detected.

In this experiment we again failed to demonstrate with any certainty that guinea-pigs receiving a preliminary small dose of toxin became immune to the Schick dose in repeated weekly Schick tests more rapidly than guinea-pigs subjected to the weekly Schick tests without the preliminary small dose of toxin.

A further experiment, recorded below, again gave inconclusive results but there was still a tendency for a few of the guinea-pigs which had received an injection followed by a rest before the retest to become immune slightly sooner than those not rested.

TABLE XV.

Initial injection.	Interval.	Immunity index.
Saline	3 weeks	22, 31, 34
0·0001 Schick dose	3 ,,	25, 49
0·001 ,,	3 ,,	38, over 45
0·01 ,,	3 ,,	17, 22
0·1 ,,	3 ,,	17, 46
1·0 ,,	3 ,,	16, 31, 39, over 52

Again we see instances of refractory guinea-pigs.

In the next experiment larger amounts of toxin were used and repeated injections given. This method was successful.

TABLE XVI.

Initial treatment.				Schick reactions.		
Number of injections given.	Interval.	Amount injected on each occasion in terms of Schick doses.	Route.	22 days.	29 days.	36 days after commencement.
17	daily	1	intracut.	+	+	–
17	,,	2	,,	+	–	–
17	,,	2	,,	+	–	–
17	,,	5	,,	+	+	–
8	alternate days	10	,,	+	+	–
8	,, ,,	10	,,	–	–	...
17	daily	1	subcutan.	+	–	–
17	,,	1	...	+	+	+
17	,,	2	,,	+	+	+
17	,,	5	,,	–	–	...
8	alternate days	10	,,	+	–	–
8	,, ,,	10	,,	+	–	–

Thus we have succeeded in rendering guinea-pigs immune (Schick negative) within 22 days by the injection of a total quantity of toxin less than 3 M.L.D.

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